OVEREXPRESSION OF HPV E6/E7 ONCOPROTEINS IS A MARKER OF PROGRESSION TO CERVICAL CANCER"

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CERTIFICATE

This is to Certify that this dissertation entitled **"OVEREXPRESSION OF HPV E6/E7 ONCOPROTEINS IS A MARKER OF PROGRESSION TO CERVICAL CANCER"** has been done by Dr. D.Kamali, Post Graduate in M.S (Obstetrics and Gynecology) under my overall supervision and guidance at Kasturba Gandhi Hospital for Women and Child Health, Institute of Social Obstetrics and Gynecology, Madras Medical College, Chennai in partial fulfillment of regulations of Tamil Nadu Dr. M.G.R. Medical University for the award of M.S.Degree in Obstetrics and Gynecology.

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DECLARATION

I, Dr. D. KAMALI solemnly declare that the dissertation titled "**OVEREXPRESSION OF HPV E6/E7 ONCOPROTEINS IS A MARKER OF PROGRESSION TO CERVICAL CANCER"** has been prepared by me. I also declare that this bonafide work or a part of this work was not submitted by me or any other person for any award, degree or diploma to any other university board either in India or abroad.

This is submitted to The Tamil Nadu Dr. MGR Medical University, Chennai in partial fulfillment of the rules and regulations for the award of M.S. degree Branch II Obstetrics and Gynaecology to be held in April 2016.

Place: Chennai **Dr. D.KAMALI**

Date:

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Lastly and most importantly, I am indebted to all my patients who willingly participated to this study.

CONTENTS

TITLE: Overexpression of HPV E6/E7 Oncoproteins is a marker of progression to cervical cancer"

INTRODUCTION

HPV E6/E7 oncoproteins initiate the development of cervical cancer. Their overexpression, is associated with significantly increased risk of CIN and cervical cancer.

HPV-HR infection
PERSISTENT

↓

DNA-HPV
INTEGRATION
in cellular genome

↓

Celullar cycle
deregulation:
E6/E7
over expression

↓

- Inactivation of P53 Tumour suppressor gene
- Blocking Celular Apoptosis
- Modulation of G Protein pathway
- Modulation of Immune System
- Induction of telomerase activity
- Modulation of Chromosomal Stability

↓

CANCER

So, E6/E7 MRNA oncoprotein test for early detection of cervical cancer.

HPV E6/E7 MRNA plus LBC test from a single specimen

Highly specific molecular test

It has Highest positive predictive value, Correlates to disease with PPV above

90% vs DNA testing PPV of 43% in cells for CIN2 plus

No false negative cases as seen in LBC test

AIM & OBJECTIVES

For early detection and treatment of Cancer Cervix Developing new molecular diagnosis method for screening and treating the patient on the E6 gene expression.

Duration of Study	One year
Study Design	Most appropriate screening & diagnostic test for Cervical Cancer all sexually active women
Sample size	50 Patients
Inclusion criteria	All sexually active women above 30 years All pap smear positive cases All VIA / VILI Positive cases All Colposcopy Cervical biopsy HPE report shows CIN-I, CIN-II and CIN-III positive cases Unmarried, all sexually active women less than 20 Years
Exclusion criteria	All pap smear Negative cases All VIA / VILI Negative cases All Colposcopy Cervical biopsy HPE report shows inflammatory changes

Methodology
(Materials and Methods)

Setting:

This study will be carried out in the Institute of Social Obstetrics, Kasturba Gandhi Hospital, Madras Medical College, Chennai – 3, in association with department of Pathology, Kasturba Gandhi Hospital, Madras Medical College, Chennai – 3.

Method:

Those who are VIAVILI Positive cases, HPE report shows CINII & CIN III going to do this study under colposcopy guided with the help of plastic spatula / endocervical brush remove the blood material, mucus and inflammatory cells, This material into the liquid fixative Solution. The suspended cells are then gently sucked onto the filter membrane and the filter is pressed onto a glass slide to form a thin monolayer, and then it is stained. The liquid can also be employed to test HPV infection, making it a cost-effective technique. The cells wash off plastic device more than wooden one, and the fixation solution contains hemolytic and mucolytic agents.

Study Population	Average age 30-45 years. Precancerous lesions occur 10-15 years earlier. Coitus before the age of 18 Years. Multiple sexual partners. Delivery of the first baby before the age of 20 Years. Multi parity with poor birth spacing between pregnancies. Poor personal hygiene. Poor socioeconomic status Immunosuppressed individuals. Women with preinvasive lesions. COC and progestogens use over 18 year period can cause adenocarcinoma of the endocervix.

OVERVIEW OF LITERATURE

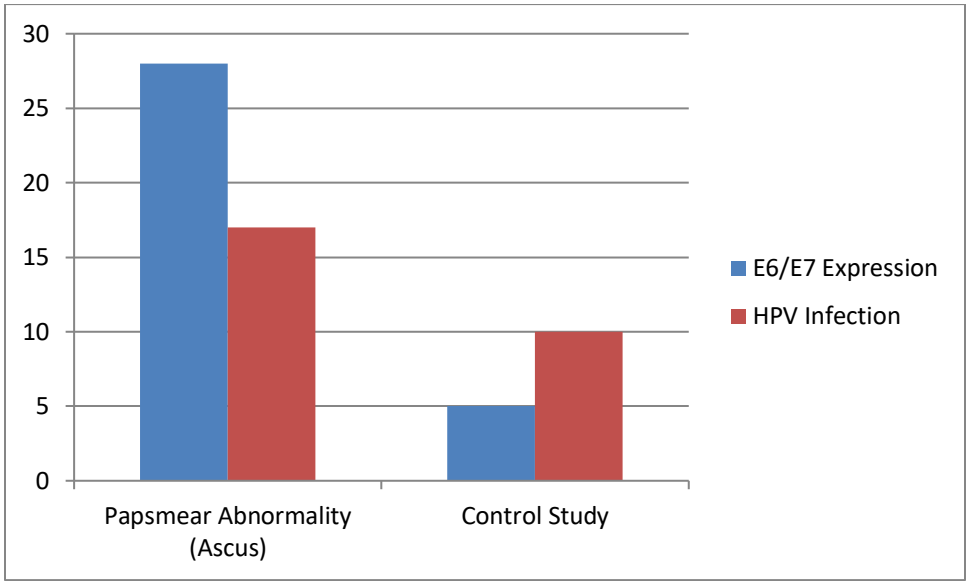
Major cause of cervical cancer is human papillomavirus which is a third most common cancer in women.

E6/E7 activities

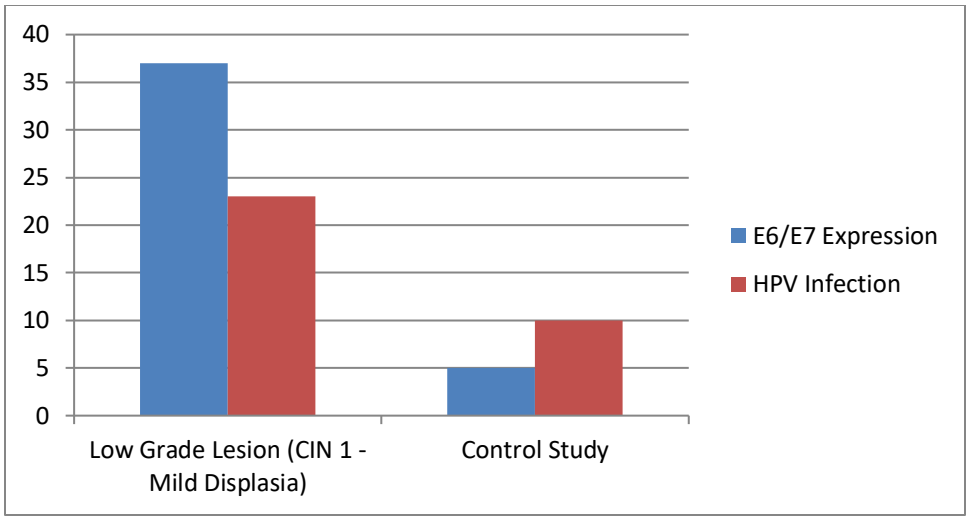
.

- E6/E7 oncoproteins degrates the cellular tumar suppressor protein p53 and induces human telomerase reverse transcriptase activity that leads to progressive cervical carcino genesis.
- E6/E7 oncoproteins involve histone acetylation in cell extract.
- E6/E7 oncoprotein involve blocking the cellular apoptosis.
- E6/E7 oncoprotein that affects the chromosomal stability.
- E6/E7 changes the polarity adhesion and Gprotein modulation.
- E6/E7 oncoprotein become overexpressed in replication of basel and para basel cells of cervix.
- E6/E7 oncoprotein promotes the cellular proliferation.
- E6/E7 oncoprotein regulates the HTERT in Keratinocytes and fibroblast.

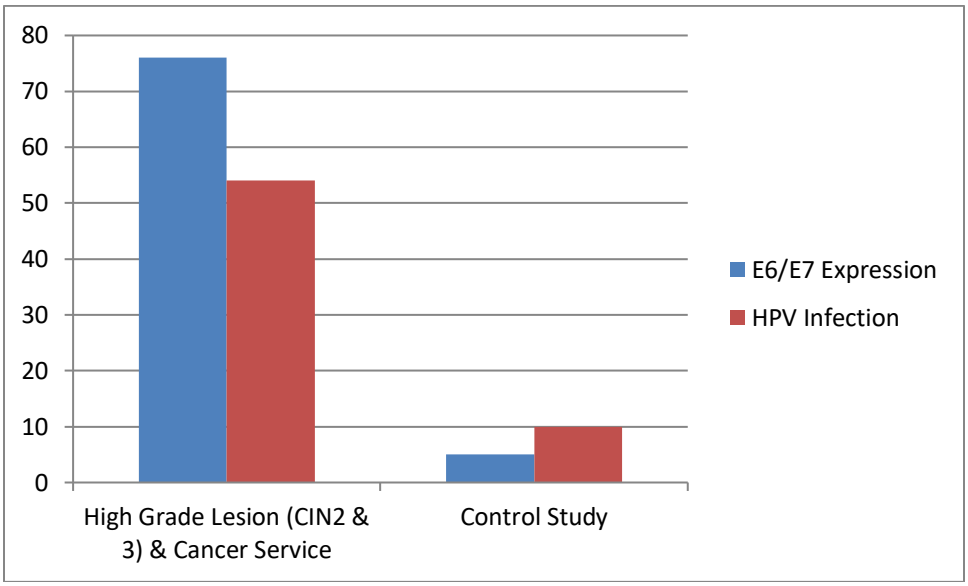
ANALYSIS OF STUDY



Bar Chart showing: E6/E7 Expression in Pap smear Abnormality (ASCUS) and Control Study.



Bar Chart showing: E6/E7 Expression in Low Grade Lesion (CIN 1 Mild Dysplasia).



Bar Chart showing: E6/E7 Expression in High Grade Lesion (CIN2 & 3) & Cancer Service and Control Study.

MASTER CHART

SNO.	Name	Age / Sex	LMP No.	VIA / VILLI	Pap smear Abnormality	E6/E7 Expression in %
1	JANAKI	23 / F	15.10.2014	Positive	ASCUS	18.80%
2	DEVI	30 / F	28.02.2015	Positive	HSIL	22.80%
3	THENMOZHI	28 / F	05.03.2015	Positive	LSIL	19.40%
4	DHANALAKSHMI	46 / F	PMS	Positive	ASCUS	28.40%
5	DEVIKA	57 / F	PMS	Positive	ASCUS	30.20%
6	SAMUTHIRAKANI	60 / F	PMS	Positive	ASCUS	30.60%
7	KARPAGAM	55 / F	PMS	Positive	LSIL	28.60%
8	JAYASEELI	45 / F	10.05.2015	Positive	ASCUS	26.80%
9	CHANDRAMMA	63 / F	PMS	Positive	HSIL	32.80%
10	BABY	40 / F	15.10.2014	Positive	HSIL	34.20%

SNO.	Name	Age / Sex	LMP No.	VIA / VILLI	HPE (Low Grade Lesion - CIN1)	E6/E7 Expression in %
1	ARUNMOZHI	35 / F	04.10.2014	Positive	CIN1	36.80%
2	MALLIKA	45 / F	05.06.2014	Positive	CIN1	34.60%
3	LATHA	30 / F	06.12.2014	Positive	CIN1	35.80%
4	VIDHYA	38 / F	06.08.2014	Positive	CIN1	30.60%
5	GOWRI	40 / F	05.06.2014	Positive	CIN1	32.40%
6	BHUVANESHWARI	36 / F	06.08.2014	Positive	CIN1	36.40%
7	RASIA SULTANNA	45 / F	05.05.2014	Positive	CIN1	28.60%
8	SIVAKAMI	38 / F	05.04.2014	Positive	CIN1	31.40%
9	KAMATCHI	45 / F	04.04.2014	Positive	CIN1	29.40%
10	VIJAYALAKSHMI	30 / F	05.08.2014	Positive	CIN1	30.10%
11	DEVAKI	36 / F	04.04.2014	Positive	CIN1	28.40%
12	SHANTHI	45 / F	04.06.2014	Positive	CIN1	29.60%
13	POONGODI	34 / F	06.06.2014	Positive	CIN1	26.80%
14	LAKSHMI	30 / F	05.12.2014	Positive	CIN1	30.10%
15	PARIMALA	30 / F	06.08.2014	Positive	CIN1	28.90%
16	KALA	32 / F	05.09.2014	Positive	CIN1	26.80%
17	PARVATHI	46 / F	06.09.2014	Positive	CIN1	29.60%
18	JAYASUDHA	30 / F	07.08.2014	Positive	CIN1	29.60%

19	KALAIVANI	43 / F	05.10.2014	Positive	CIN1	29.80%
20	GOKILA	63 / F	PMS	Positive	CIN1	32.60%

SNO.	Name	Age / Sex	LMP No.	VIA / VILLI	HPE (High Grade Lesion - CIN2 & 3)	E6/E7 Expression in %
1	RAJAMMAL	48 / F	06.12.2014	Positive	CIN3	46.20%
2	TAMILSELVI	48 / F	PMS	Positive	CIN2	42.80%
3	DEVI	38 / F	05.12.2014	Positive	CIN2	41.90%
4	JEENIRA	40 / F	05.09.2014	Positive	CIN2	40.80%
5	POONGAVANAM	62 / F	PMS	Positive	CIN3	46.80%
6	KANAGI	39 / F	05.10.2014	Positive	CIN2	42.80%
7	SARASWATHI	45 / F	PMS	Positive	CIN3	48.20%
8	ANJALAI	40 / F	05.10.2014	Positive	CIN3	49.60%
9	CHANDRA	50 / F	PMS	Positive	CIN3	46.80%
10	NAVANEETAM	41 / F	05.12.2014	Positive	CIN3	45.80%

SNO.	Name	Age / Sex	LMP No.	VIA / VILLI	HPE Cancer cervix	E6/E7 Expression in %
1	KALA	55 / F	PMS	Positive	SQ CELL CA	79.60%
2	THIRUMANGAI	38 / F	05.10.2014	Positive	SQ CELL CA	68.90%
3	SARASAMMAL	58 / F	PMS	Positive	SQ CELL CA	73.80%
4	DHAMAYANTI	50 / F	PMS	Positive	SQ CELL CA	76.80%
5	PARVATHI	40 / F	06.10.2014	Positive	SQ CELL CA	78.60%
6	EASWARI	49 / F	PMS	Positive	ADENO CELL CA	68.90%
7	RATINAMMAL	40 / F	05.08.2014	Positive	SQ CELL CA	65.80%
8	ESAKKIAMMAL	50 / F	PMS	Positive	SQ CELL CA	70.10%
9	LAKSHMI	37 / F	05.06.2014	Positive	SQ CELL CA	68.90%
10	DHANALAKSHMI	44 / F	05.08.2014	Positive	ADENO CELL CA	65.80%

RESULT

In this study deducted the E6/E7 oncoprotein expression positivity in high grade cervical lesion shows around 75% to 80% , low grade cervical lesion shows 40% to 45% and Atipical spamus cell lesion shows around 20% to 25%.

CONCLUSION

This study has done in our hospital that over expression of HPV E6/E7 ONCOPROTEIN is a marker of progression to cervical cancer. The result were compared based on HPE grading of lesion, ca cervix and High Grade lesion gave 70 to 80% of expression of E6/E7 ONCOPROTEIN. According to my study there is earlier diagnostic / screening test for ca cervix, so identify the lesion earlier and to decide the modality of treatment earlier. My study findings are suggested that HPVE6/E7 ONCOPROTEIN expression could be used in diagnosing high grade cervical lesion and predictive tool for screening of low grade cervical lesion.

REVIEW OF LITERATURE

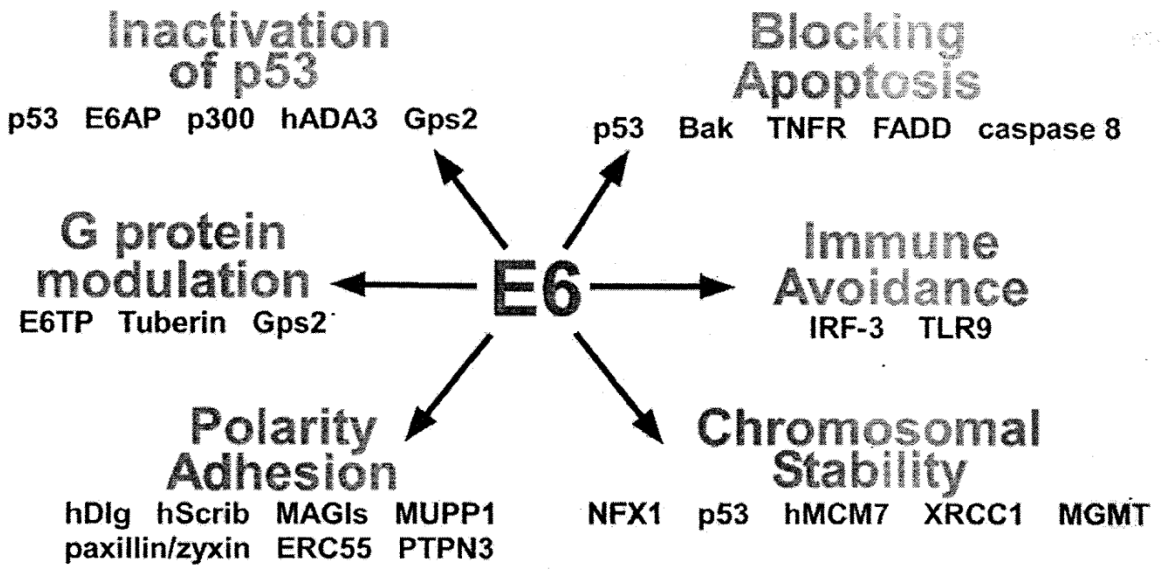


Figure 2.
Binding Partners of E6. E6 alters numerous cellular pathways through the binding of other proteins.

Papillomavirus E6 proteins

The papillomavirus are small DNA viruses that encode approximately eight genes, and require the host cell DNA replication machinery for their vital DNA replication. Thus papillomaviruses have evolved strategics to induce host cell DNA synthesis balanced with strategics to protect the cell from unscheduled replication. While the papilomavirus E1 and E2 genes are directly involved in vital replication by binding to and unwinding the origin of replication, the E6 and E7 proteins have auxiliary functions that

promote proliferation. As a consequence of disrupting the normal cheek points that regulate cell cycle entry and progression, the E6 and E7 proteins play a key role in the oncogenic properties of human papillomaviruses with a high risk of causing anogenital cancers . As a consequence E6 and E7 of HPVs are invariably expressed in cervical cancers. This article will focus on the E6 protein and its numerous activities including inactivating blocking apoptosis, activating telomerase, disrupting cell adhesion, polarity and epithelial differentiation, altering transcription and reducing immune recognition.

Structure of E6

All papillomaviruses encode an E6 open reading frame immediately downstream of the non coding region . E6 proteins are approximately 150 amino acids containing two CX_2C-X_{29}-CX_2Czinc- like fingers joined by an inter domain linker of 36 amino acids, flanked by short amino and car boxy terminal domains of variable lengths. The E7 protein contains similarly

spaced zinc- like finger leading to the hypothesis that the E6 and E7 genes may have arisen from duplication events for one of these core motifs. A number of papillomavirus E6 and E7 proteins have been shown to bind zinc through the coordination of the cysteine residues . Attempts to obtain the crystal structure of E6 have been plagued by the tendency of E6 to form insoluble aggregates. Recently the solution structure of the C- terminal half of HPV 16E6 was solved by nuclear magnetic resonance , and a model for the whole protein was proposed. The two zinc finger domains face each other symmetrically in a pseudodimeric fashion with the inter domain forming a helix that contributes to the rigidity of the two domains. Each domain consists of a three slandered beta sheet and two short helices. The N and C terminal domains are of variable lengths and sequences. The proposed structure indicated that many of the mutations that have been made to map functions of E6 likely disrupted the overall structure, rather than specific protein- protein interactions.

Although E6 may dimerize at high salt and protein concentrations, it is thought to be monomeric at physiologic conditions. It has been difficult to study the expression of endogenous E6 proteins as they are expressed at low levels and few sensitive antibodies exist, however E6 is thought to be largely nuclear, though some fraction of E6 on coding residues 1-41 of 16B6 inactivates the functions of full- length E6, which is proposed to be due to its ability to bind to the interface of the N- and C- terminal halves of E6. No enzymatic activities have been reported for E6, and although the HR E6 proteins have been reported to bind specifically to four-way DNA junctions, most of the activities of E6 are thoughts to be mediated by protein proteins interactions.

E6 binding motifs

The first protein that was shown to interact with E6 was E6 associated protein, an E3 ubiquitin cascade functions to target proteins for proteasomal degradation by means of adding multiple ubiquitin monomers

to the protein destined to be destroyed. E1 proteins activate the ubiquitin monomers that are subsequently transferred by E2 conjugating enzymes to an E3 ubiquitin ligase, which confers target specificity E6AP is the founding member of the HECT – domain family of ubiquitin ligases, a group of related proteins with homology too E6AP C- terminal domain involved in ubiquitination of bound substrates, and divergent N- termini that mediate substrate specificity E6AP forms a complex with both E6 and target proteins leading to ubiquitination of the target protein and subsequent proteasome mediated degradation. The most well studies E6/E6AP target is the tumor suppressor though other proteins are also targeted by this complex. Numerous studies have identified residues of the E6 protein that diminishes binding to E6AP, but recently most of these mutations have been predicted to disrupt the global integrity of E6. Moreover, mutations of numerous surface exposed residues of E6 did not eliminate its binding to E6AP. However, the motif on E6AP that binds E6 has been extensively characterized and is referred to an LXXLL motif A number of other

proteins also bind to E6 by way of LXXLL motif including ERC-55, IRF3, PAXILLIN AND TUBERIN . These motifs are leucine rich amphipathic helices with limited substitution of hydrophobic residues for leucines and at least one negative charge in an X position. The sequence of E6AP that interacts with E6 is ELQULLGE Bending to proteins with an LXXLL motif is a conserved property of the proteins of n numerous papillomaviruses, as E6AP binds to both high and low risk genus alpha HPV's at least some genus beta HPV's and bovine papillomanvirus type 1.

HR HPV E6 proteins all have a motif designated as XT/SXV at their C-tennini. This motif on the E6 protein mediates binding to specific domains on cellular proteins known as PDZ proteins PDZ domains are approximately 90 amino acid stretches found in a wide variety of proteins and are named after the first letters of three proteins which were first discovered to share the domain, post synaptic density protein , Drosophila disc large tumor suppressor and zoniula occludents –I protein. Among the

PDZ proteins reported to bind E6 are human homologs of bscriib, a homolog of the Drosophilla scribble protein Hutibregate. Membrane Associated Guanylate kinase homology proteins with an Inverted doimain structure. MUPPI, a multi PDZ protein and PTPN3, a membrane associated tyrosine phosphatase. Mutational analyses have demonstrated the importance of the XS/TV motif in binding, but it is possible that other residues of E6 also bind and confer specificity for which PDZ proteins and which PDZ domains are bound. The concurrent binding of E6 To E6AP and PDZ proteins can target PDZ proteins for degradation, though other ubiquitin ligases have also been implicated.

Other proteins such as p53, Bak, p300/CBP, hADA3, nfxi, Gps2, FADD, and procaspase 8 have been reported to bind various E6 proteins, but they lack both LXXLL and PDZ domains .They may bind to E6 through as yet underfined motifs, or indirectly through binding to E6AP or other E6 associated proteins.

INACTIVATION OF p53

One of the most well studied interacting proteins of E6 is the p53 suppressor, a DNA site specific transcription factor, and one of the key signaling coordinators in the cell following genotoxide or cytotoxie stress. Normally present in low levels and transcriptionally inactive, cellular damage triggers an increase in p53 protein levels and activation via post translational modifications. Once activated, p53 functions to initiate pathways for DNA repair, cell cycle arrest and/ or apoptosis, based upon the type and extent of damage. The importance of p53 in orchestrating the cellular response to cytotoxie agents is exemplified by the observation that approximately one-half of all human cancers harbor mutations in the p53 gene. These mutations impair the ability of p53 to trigger the appropriate signaling pathways to repair the damage or trigger cell death in cases where the damage is beyond repair. This in turn allows for replication of

damaged DNA, and survival of cells with deleterious mutations that would normally be eliminated.

Importantly, in addition to genotoxic and cytotoxic damage, p53 is also activated upon improper stimulation of DNA synthesis, such as that induced by HPV infection. Both HR and LRHPVs infect cells in the basal layer of the epithelium, however viral replication occurs only in differentiating cells- which would have normally exited the cell cycle and turned off their cellular DNA synthesis machinery HPVs must therefore stimulate DNA synthesis in these cells, while concomitantly inhibiting the normal cellular response of activating p53. Importantly, unlike most other cancer cell types, cervical cancers generally harbor wild- type p53. HPVs have evolved a number of mechanisms to block p53 function in the infected cell through the actions of the E6 oncoprotein to this way sour understanding of E6 highlights the functions of p53 in normal cells, and demonstrates the mechanisms by which a regulatory checkpoint can be

countered during vital infection and or cancer progression via protein degradation , mislocalization and modification.

The principle mechanism by which HR HPVs inactivate p53 is by inducing its degradation through the ubiquitin proteasome pathway. Normally p53 protein levels are regulated by the Mdm2 E3 ubiquitin ligase. However Mdin2-mediated degradation of p53 is inhibited during vital infection and other stress conditions, allowing for stabilization of p53 protein levels and subsequent activation. Instead, HR HPV E6 induces p53 degradation by forming a complex with E3 ubiqutin ligase, E6AP .

E6AP is unable to bind and induce its degradation. Instead, E6 must first bind to the n terminal substrate recognition domain of e6ap before p53 can be bound and ubiquitnated. Both HR and LR HPV E6 proteins have been shown to interact with p53 however they differ with respect to the domains of p53 with which they interact. While both HR and LR E6

proteins cab bind to the p53 C- terminus, only HR E6 proteins are capable of binding to the core region of p53. It is this binding of the core region that is required for p53 degradation by HR E6.

Although stimulation of p53 degradation mediates a considerable block to p53 function, not all of the p53 within E6 expressing cells is degraded. Moreover, as mentioned above, the LR genus alpha HPV, and the genus beta HPV E6 proteins do not degrade p53 and therefore have evolved different mechanism of circumventing p53 growth suppression. In part, infection with these papilomaviruses may induce a p53 response less robustly that the HR HPVs. Additionally, some assays have shown that expression of both HR and LR E6 proteins can abolish p53 mediated transcriptional activity. It has been shown that E6 can mediate these effects through a number of mechanisms, including inhibition of p53 binding, aberrant p53 localization, and post translational modification of the p53 protein.

First it has show that E6 interaction with p53 can inhibit the binding of p53 to its site specific DNA sequences . The level of inhibition was found to correlate with the affinity that each E6 protein has for p53 thus 16E6 shows the highest level of site specific binding inhibition, 31E6 and 18E6 show intermediate levels of inhibition, and 11E6 shows the least, albeit still detectable inhibitory effect. Moreover, it was later shown that E6 association with p53 can induce a conformational change in the p53 protein, which in turn leads to an inhibition of p53 binding to DNA, or a dissociation between p53 DNA complexes that have already been formed . Importantly, these inhibitory effects were shown to correlate with the ability of different E6 proteins to inhibit p53 transactivation, and were shown to be independent of E6/E6AP – mediated p53 degradation.

The second proposed mechanism by which E6 my be able to inhibit p53 signaling independent of protein degradation is by means of

sequestration of p53 in the cytoplasm. This has been hypothesized to be a result of either masking of the nuclear localization signal on the p53 terminus due to E6 binding of p53 or an enhancement of p53 nuclear export. As both HR and LR E6 proteins are able to bind to the C- terminus of p53 The second proposed mechanism by which E6 my be able to inhibit p53 signaling independent of protein degradation is by means of sequestration of p53 in the cytoplasm. This has been hypothesized to be a result of either masking of the nuclear localization signal on the p53 terminus due to E6 binding of p53 or an enhancement of p53 nuclear export. As both HR and LR E6 proteins are able to bind to the C- terminus of p53 masking the p-53NLS is an attractive possibility. Evidence for E-6 mediated mislocalization of p53 NLS is an attractive possibility. Evidence for E6- mediated mislocation of p53 has been demonstrated from experiments using cervical cell lines in which E6 mediated p53 degradation was blocked in these cells, even though p53 levels returned to normal, correct nuclear localization of p53 was perturbed.

The third mechanism employed by E6 to inhibit p53 activity is its abrogation of the transactivation of p53 responsive genes via interaction with either the CBP/p300 histone acetylinaferases. Following DNA damage p300 is known to acetylate p53, thus enhancing its ability to bind to specific DNA sequences in the promoters of p53 responsive genes, and in turn up regulating their transcription. The E6 proteins have been shown to bind to p300, and this interaction inhibits p53 acetylation at p300 dependent sites, leading to decreased expression from a p53 responsive luciferase reporter. Both HR and LR E6 proteins have been shown to bind to p300, although the HR E6 proteins appear to bind with a higher affinity In-vivo studies have reported that only the HR E6 proteins prevent p300 transactivation of p53 responsive genes . However an in-vitro analysis using chromationized templates demonstrated that LR HPV 11E6 was also capable of inhibiting p53 transactivation. This study also demonstrated that while E6 mutants deficient in E6AP binding were capable of inhibiting p53 mutants defective

in binding to either p53 or p300 were unable to elicit this response. Thus a complex between E6, p53 and p300 appears to be required to block transactivation.

Similarly, HR E6 proteins are also able to block p53 activation by interacting with another histone acetyltransferase, HADA3 . HADA3 is the human homolog of the yeast transcriptional activator y,ADA3, which functions as an essential component of the ADA transcriptional co activator complex. However, unlike with p300, E6 interaction with hADA3 results in hADA3 degradation . This degradation has been shown to abrogate both p53 and retinoic X receptor alpha mediated transactivation.

Finally, recent evidence suggests that E6 may also inhibit p53 activation by blocking the p14/ARF pathway following most cellular stresses p53 is activated due to cellular signals that prevent its interaction with and degradation by Mdm2. However p53 can also be activated during

oncogenenic stress by a mechanism involving direct inhibition of Mdm2 ubiquitin ligase activity through its association with ARF . 16 E6 has been shown to inhibit p14/ARF dependent activation of p53 without inactivation of the p53 dependent DNA damage response, and in a manner that is not dependent on E6- mediated p53 degradation. Interestingly, one mechanism by which this has been proposed to be facilitated is through the degradation of hADA3.

Modulation of G-Protein signaling

As described above, E6 is able to modulate transcription of p53-dependent genes either by degradation of p53 or via interaction with the p300 and hADA3 transactivators. In addition, yeast two-hybrid experiments from other cellular signaling pathways as well, highlighting the ability of the E6 oncoprotein to act as a diverse modulator of host cell signaling. With respect to G-protein signaling. E6 has been shown to interact with three different proteins. First, Hr E6 was shown to bind and degrade a novelprotein termed E6-targeted proteins 1 in an E6AP

dependent manner. E6TP1 has homology to GTPase activating proteins (GAPs) for Rap, and recent experiments demonstrated that E6TP1 does indeed harbor GAP activity for Rap 1 and Rap 2, Importantly, E6 mediated degradation of E6TP1 enhances the GTP-loading of RAP, thus supporting a role for the RAP small G-protein pathway in E6-mediated oncogenesis. Another protein with GAP activity, tuberin, was also shown to be bound and degraded by E6. Tuber in functions in the harmatin-tuberin complex, which exhibits GAP activity toward the small G-protein Rheb, and the complex is a critical negative regulator of m TOR signaling (reviewed in Huang and Manning, 2008). As this pathway is an important mediator of cell growth, it highlights yet another mechanism by which E6 can modulate the regulation of such processes. Finally, E6 from both HR and LRHPVs was shown to bind and degrade Gps2. Gps2 is involved in suppressing G-protein mediated signaling pathways, C-Jun N-terminal kinase activity, and known to stimulate transcriptional activation by the BPV1 E2 protein. As seen with BPV, Gps2 was found to stimulate transcription from HPV promoters. Moreover, HR

E6-mediated degradation of Gps2 was shown to suppress this transactivation of the HPV early promoter. As Gps2 is known to interact with an positively regulate p300, the implications of Gps2 degradation may extend beyond that of transcriptional control of HPV encoded genes, however this remains to be determined.

Modulation of Immune Recognition

E6 has also been shown to modulate transcription of genes whose protein products are involved in innate immunity. HR E6 has been shown to interact with two proteins that are part of the innate immune response to viral infection; Interferon regulatory factor-3 and Toll-like receptor 9, IRF-3 becomes activated by dsRNA or viral infection, and this activation leads to transcription of Interferon-B. 16E6 interaction with IRF-E has been shown to inhibit its transactivation ability, and thus prevents the induction of IFN-B following viral infection. TLR9 becomes activated by viral or bacterial dsDNA derived CpG motifs, and induces cytokine

production as a means to defend the cell against the invading organism. Exogenous expression of 16E6/E7 has been shown to inhibit TLR9 transcription, leading to a functional loss of TLR9 signaling pathways within the cell. Similar results were seen in the HPV 16 positive cervical carcinoma cell lines CaSki, SiHa and HeL-a, demonstrating that this may indeed contribute to HPV-mediated carcinogenesis.

Blocking apoptosis

One of the main consequences of E6 degrading or blocking p53 function is to inhibit apoptotic signaling that would otherwise eliminate the HPV infected cell. However, p53 independent apoptotic signals can also be employed to eliminate abnormal cells, and E6 has been shown to block apoptosis in cells and mice lacking p53. There are two major apoptotic pathways that can be triggered by different stresses – the extrinsic pathway, and the intrinsic pathway. Interestingly, the E6 protein has been shown to disrupt both pathways to

facilitate a cytoprotective environment and prevent cell death, thus highlighting the critical signaling events that a cell undergoes following exogenous or endogenous stress.

The extrinsic apoptotic signaling pathway can be induced during viral infection as part of the host response, and is triggered by extracellular signals that induce the activation of “death receptors” on the cell surface. These receptors are members of the tumor necrosis factor receptor family and include TNF receptor 1, Fast CD95 and the TNF-related apoptosis inducing legend receptors DR-4 and DR-5. Upon binding to their cognate legend, these receptors trimerize and recruit adapter molecules such FADD, TRADD and initiator caspases such as caspase-8 and caspasc-10 to their cytoplasmic death domains, thus forming the death inducing signaling complex. The DISC is involved in activating caspase 8, which in turn cleaves and activates downstream executioner caspases. The executioner

caspases target and cleave downstream substrates such as poly polymerase and Lamin B.

E6 has been shown to inhibit extrinsic apoptotic signaling at each of the early stages, by interacting with TNFR-1, FADD and caspase-8, 16E6 was shown to directly bind to the death receptor TNFR-1, an interaction that inhibited TNFR-1 association with the TNFR1-associated death domain adapter molecule, and blocked TNFR-1 DD mediated apoptosis. Importantly, 16E6 has also been shown to block apoptosis normally triggered by TNF, the cognate legend for TNFR-1. In addition to the TNF pathway, it has also been shown that 16E6 is capable of inhibiting apoptosis stimulated by both the FAS and the TRAIL pathways. This inhibition is mediated by E6 binging to and degradation of both the FADD adapter protein and the effector caspase, caspase-8. As FADD and caspase-8 are key components to apoptotic signaling through all of the death receptors, this mechanism of cytoprotection demonstrates how the E6 oncoprotein is able to

exploit one or two proteins as a means to block multiple signaling pathways. Importantly, these effects were all seen using the HR 16E6 oncoprotein, and it is unknown whether LR or genus beta HPVE6 proteins function in a similar manner with respect to blocking extrinsic apoptotic pathways. As the binding of FADD is not dependent on the conserved PDZ domain of HR E6, but rather a novel domain, it is possible that other E6 proteins may inhibit these extrinsic pathways.

The intrinsic apoptotic pathway is involved in sensing apoptotic signals that originate from within the cell, such as DNA damage, oxidative stress, starvation and those mediated by chemotherapeutic drugs. These stresses activate a number of pathways that converge on the mitochondria, which acts as a hub to sese to balance of pro- and anti-apoptotic signals and facilitate downstream apoptotic signaling when this balance is upset. When the cell senses intrinsic stress, pro-apoptotic BH3- only proteins become activated and abrogate the function of anti-apoptotic proteins. This allows for the formation of pores in the

mitochondrial membrane comprised of prop-apoptotic Bax or Bak, and subsequent release of mitochondrial inner membrane proteins such as cytochrome c, apoptosis inducing factor, endonuclease G, Smac/Diablo and Htr/Omi. These mitochondrial proteins form the “apoptosome”, an apoptotic signaling complex that, like the DISC from the extrinsic pathway, result in the cleavage of caspase 3 and caspase 7, and ultimately cell death.

The E6 oncoproteins from both HR and LR HPVs have been found to block intrinsic apoptotic signaling primarily by interacting with only one protein – BAK. Both HR and LR E6proteins from genus alpha and beta HPVs have been shown to bind BAK, and induce its proteasomal-dependent degradation. While E6AP has been shown to play a role in BAK degradation, it has also been proposed that this may not be a universal mechanism for all of the HPV types, thus the involvement of other E3 ubiquitin ligases in this process is an area of interest. Whether BAK degradation is constitutive or induced following cell stress is also a topic of

controversy. Recent evidence has shown that BAK degradation is not constitutive, but rather occurs only after apoptotic signals have been initiated, indicating that a BAK conformational change and/or dissociation from its anti-apoptotic partner MCL-1 may be necessary for its interaction with E6 and E6AP. Other studies have found constitutively lower levels of total BAK protein in E6 expressing cells. Regardless of whether BAK degradation is constitutive or induced, the overall effect has been shown to be a block the release of cytochrome c, AIF and Omi from the mitochondria, preservation of mitochondrial integrity, and disruption of the cleavage of effector caspases. A final area of controversy surrounds the ability of E6 to perturb increase in the anti-apoptotic Bc1-2 protein. Another group demonstrated that 5E6 and 8E6 expression leads to a decrease in the levels of Bar protein expression. A third study examined the levels of NOXA, Puma, Bc1-xL, Mc1-1, Bc1-2 and BAX, and found none of these intrinsic apoptotic signaling proteins to be perturbed in E6-expressing cells.

Importantly the extrinsic and intrinsic pathways are not isolated. Caspase 8 can be activated during intrinsic apoptotic signaling via an amplification loop mediated by caspases 3 and 7. Likewise, mitochondrial signaling can be triggered during activation of the extrinsic cascade, via caspase 8 mediated cleavage of the BH3-only protein Bid. These mechanisms are through to help amplify apoptotic signaling once signals have been detected. Thus, by E6 targeting both intrinsic and extrinsic signaling, it not only protects an infected cell from multiple apoptotic stimuli, but also protects the cell from cross activation between these two pathways. Moreover, E6 has been shown to interact with proteins that are involved in apoptotic signaling at the crossroads where the intrinsic and extrinsic pathways join, downstream of the effecter caspases. HPV 16E6 has been shown to upregulate the expression of two inhibitor of apoptosis proteins, c-IAP2 and suvivin. IAPs are proteins that can bind to and inactivate the executioner caspases, caspase-e and caspase-7. c-IAP2 up regulation appears to be due to E6 mediated activation of NF kappa B, a well known anti-apoptotic signaling

molecule. As NF kappa B has other well known cytoprotective effects, it is possible that other NF kappa B responsive proteins play a role in E6 mediated cytoprotection as well. Finally, 16E6 dependent manner. However other studies have reported increased levels of c-MYC in E6 expressing cells or no change in the level of c-MYC, thus the overall role that C-MYC may play in E6 mediated cytoprotection is uncertain.

Induction of telomerase activity

Expression of HR HPV E6 protein in concert with HR HPV E7 protein can immortalize epithelial cells in culture and one critical cellular target is telomerase. Understanding how E6 affects telomerase shines a spotlight on a critical target for oncogenic progression and avoidance of cellular senescence.

As linear DNA is replicated in each cell division, approximately 150-200 nucleotides at the 3’ end of chromosomes is lost due to the directional

amplification of DNA. To avoid losing critical genetic information on chromosomes and to prevent recombination at the termini, their ends are capped with repetitive telomeric DNA approximately 10-12 kilo bases in length and proteins collectively named sheltering. The protection of genetic material within chromosomes is important, as is the folding of telomeric DNA into a T-loop to avoid DNA damage signals and senescence or apoptosis signals. As telomeres shorten with each cell division, the age of a cell is clearly marked in time. When telomeres become critically shortened, cells are signaled to senesce; if they do not, there can be catastrophic DNA damage, including anaphase bridges and double strand DNA breaks.

Telomerase is a ribonucleoprotein that extends to telomeric ends of linear chromosomes in eukaryotes. Normally quiescent in somatic cells, telomerase is active in stem cells and during embryonic development. The RNA component of telomerase, TERC, is the template for the repetitive DNA in telomeres, TTAGGG in

humans. Dyskerin is an additional key protein subunit of telomerase, and HTERT is the catalytic subunit of telomerase. The expression level of HTERT is proportionate to the enzymatic activity of telomerase in cells, and in the majority of immortalized cells and cancers HTERT is detected. If telomerase is not active in immortalized cells or in cancers, telomeric DNA is extended using the alternative lengthening of telomeres pathway of homologous end joining. So, the protection of telomeric DNA to avoid signals of cellular senescence or catastrophic chromosomal damage is key to cellular dysregulation in cancers and must be controlled in normal, differentiated somatic cells.

In 1996 it was found that HR HPV E6 protein could activate telomerase in epithelial cells and less than ten later it was found that E6AP was critical for HTERT regulation by E6. HR E6 binds the endogenous E6AP protein and utilizes it in its activation of HTERT and telomerase. Knockdown of E6AP decreases the telomerase activity triggered by HPV 16E6, as well as genus beta HPV E6 types 38

and 8. In one study, mutants of PV 16E6 that cannot bind E6AP, when expressed in epithelial cells, were also unable to induce HTERT transcription, however, another study using E6 mutants did not find a requirement for E6AP in HTERT induction.

The oncogene c-MYC has been shown to activate HTERT transcription. The heterodimer c-MYC/Max binds two E-box sequences in the core proximal promoter of HTERT. The HR E6 protein required the two proximal E-box sequences to activate HTERT, as mutations in the E-box sequence blunts the activation of HTERT by E6 in luciferase assays. In vivo, E6 c-MYC, and E6AP are found at the HTERT promoter and although there is no strong evidence for significant total increases in protein levels of C_MYC nor differences in c-MYC levels at the HTERT promoter with E6 expression in Keratinocytes, c-MYC is important in HPV associated and non-HPV associated activation of HTERT, The interaction of HPV E6 with E6AP and c-MYC helps define the role of c-MYC in

HTERT activation in keratinocytes and differentiate the regulation of HTERT in keratinovytes and fibroblasts.

Upstream stimulatory factors also bind to E-box sites and disrupt binding of c-MYC/Max. Although USFs bind with less affinity to E-boxes, USF1 and USF2 are more abundant than c-MYC/Max and can compete for these sites. USF1 and USF2 have been reported to occupy the HTERT remoter in keratinocytes, and with E6 expression the amount of USF1 and USF 2 at the promoter is reduced.

GC-rich sequences are founds throughout the HTERT promoter, and Sp1 binds to five GC-rich sites flanked by the two critical E-boxes. Mutations within these sites reduce the basal expression detected from the HTERT promoter, and the increases in HTERT promoter driven expression are seen in HPV E6 cells. Therefore, the effects of HPV E6 on HTERT expression point to an additional

transcriptional activator of HTERT found commonly in keratinocytes and recruited by HPV E6 for its activation of telomerase.

Transcriptional activators and RNA polymerases all require access to their DNA sequences, like c-MYC/Max to their E-boxes and Sp1 to its GC-rich sites. Acetylation of histones on which DNA is wound opens the structure of chromatin to these proteins. HPV E6 induces histone acetylation at the HTERT promoter, and this acetylation depends on E6AP. With continued passage of HPV E6 cells in culture, the acetylation of the HTERT promoter increases, and knockdown of E6AP reduces this acetylation. Thus HPV E6 with E6AP adds to the evidence of direct transcriptional gene regulation through transcriptional activators or repressors binding to cis elements in promoters, as well as epigenetic regulation of gene expression, all through its effect on HTERT.

As E6/E6AP targets p53 for polyubiquitination and degradation, it also was hypthesized that E6/E6AP may target a yet unknown transcriptional repressor at the HTERT promoter for polyubiquitination and degradation. In 2004, NFx1-91 was identified as a transcriptional repressor of HTERT in a yeast two hybrid screen with HPV 16E6 and E6AP. NFX1, or nuclear factor binds to the X1 box, was originally identified as an MHC class II gene repressor. Two splice variant isoforms are expressed in epithelial cells, with NFX1-91 referring to its kilodalton mass. In keratinocytes, NFX1-91 binds to a X1 box sequence in the HTERT promoter, recruiting to transcriptional co-repressor mSin3A and histone deacetylase activity to shut off HTERT expression. The protein-protein interaction of NFX1-91 with the heterodimer HPV E6/E6AP identified a new endogenous transcriptional repressor of HTERT important in the regulation of telomerase in epithelial cells.

Like p53, NFX1-91 is polyubiquitinated and targeted for degradation by 16E6/E6AP. The level of NFX1-91 in HPV 16E6 expressing cells is reduced, as is its

occupancy at the HTERT promoter. Concomitantly, histone acetylation increases at the HTERT promoter, though the acetylase has yet to be identified.

To fully induce HTERT expression and telomerase activity in kertinocytes, HPV E6/E6AP requires expression of NFX1-123, the other splice variant of NFX1. Unlike NFX1-91, NFX1-123 augments HTERT promoter driven expression by HPV E6/E6AP. NFX1-123 interacts with cytoplasmic poly (A) binding proteins, which bind to the poly (A) tail of mRNA and increase protein expression through nuclear-to-cytoplasmic transcript shuttling, translational machinery recruitment, and mRNA stabilization. The PAM2 of NFX1-123 is required for the interaction with cytoplasmic poly(A) binding proteins, and this motif is critical for the increase in HTERT expression when NFX1-123 is over expressed in E6 expressing keratinocytes. This interaction between HPV E6/E6AP, NFX1-123, and cytoplasmic poly(A) binding proteins, hints at additional evidence of HTERT regulation beyond transcriptional regulation.

Mediating Chromosome Stability

In addition to increasing the expression of HTERT, E6 also interacts with other proteins involved in maintaining chromosomal stability within the HPV infected cell. First, E6 from both HR and LR HPVs have been shown to interact with the human mini chromosome maintenance 7 protein, although binding by HR E6 proteins appears to be stronger than that by LR E6 proteins. Moreover, 18E6 was shown to mediate MCM7 degradation via E6AP and proteasomal involvement. As MCM7 is involved in licensing DNA replication to ensure a single round of DNA replication per cell cycle, it is thought that E6 interaction with and/or degradation of MCM7 may lead to chromosomal abnormalities in HPV infected cells. E6 has also been shown to interact with two proteins involved in single strand DNA break repair – XRCC1 and O(6)-methylguanine-DNA methltransferase. XRCC1 was shown to be bound by HPV 1, 8 and 16E6, and this interaction reduced the ability of XRCC1 to repair meth1 methane sulfate induced

DNA damage. E6 interaction with MGMT induces its proteasomal-mediated degradation via E6AP, which has been hypothesized to sensitize HPV-infected cells to alkylating DNA damage. Finally, HR E6 mediated p53 loss inactivates the G1 checkpoint. Prolonged proliferation in the absence of p53 can lead to the loss of the G2 checkpoint, which can result in aneuploidy. Together, these interactions may lead to increased genomic instability and accelerate the progression to carcinogenesis.

Disrupting cell adhesion, polarity and epithelial differentiation

Basal cells of squamousepithlium attach to the extracellular metric (ECM) of the basement membrane and receive signals allowing their proliferation. Once daughter cells detach from the basement membrane and migrate to suprabasal layers, proliferative signals cease and markers of epithelial differentiation are expressed. The establishment of cell ECM adhesion, cell, cell contact, cytoskeletal organization and apicobasal polarity of epithelial cells is tightly regulated to

assure regulated proliferation and differentiation. E6 proteins, particularly of HR HPVs, disrupt many of these processes to allow proliferation of differentiated cells and inhibition of terminal differential to support viral replication.

Both PAXILLIN and ZYXIN are focal adhesion molecules that are involved in tethering the cellular cytoskelton to the ECM and transmitting signals along the actin network from the ECM to the nucleus. E6 from diverse papilomaviruses have been shown to bind to these proteins resulting in the disruption of action fibers and a failure to maintain proper cell structure.

HSCRIB, a PDZ domain containing protein, is also involved in epithelial tight junctions, mediating the adhesion of basal cells to the ECM. It functions as a tumor suppressor that negatively regulates proliferation. HR HPV E6 proteins bind to HSCRIB, and at in some cell types have been shown to mediate its degradation. Similarly, HDLG is a PDZ domain containing protein involved in epithelial tight

junctions, cell, cell junctions and epithelial polarity, functioning as a tumor suppressor. HDLG was the first PDZ protein shown to bind to HR E6 proteins. The MAGI proteins and MUPP1 also play role in maintain epithelial cell junctions, negatively regulating cell proliferation and in signal transduction from the cell membrane. Likewise they bind HR HPV E6 proteins and disrupt regulation of epithelial proliferation. Recently it has been shown that PTPN3, a membrane-bound tyrosine phosphatase that regulates growth factor receptors is also a PDZ protein that binds and is disrupted by E6.

Whether HR HPV E6 proteins bind and target all those PDZ proteins for degradation in vivo is controversia;. When the HPV 31 genome was transfected into keratinovytes Western analysis did not reveal any significant changes in the levels of PDZ proteins and in organotypic raft cultures, immunohistochemical analysis failed to identify substantial changes in the differentiation-dependent membrane localization of HDLG proteins. Instead, deletion or mutation of PDZ

domain-binding motif of E6 impaired the growth rate of cell lines harboring the mutant genomes and reduced the viral copy number compared to cells transfected with wild-type genomes. The results suggested that binding of E6 to PDZ proteins modulates the early viral functions such as proliferation and maintenance of the viral copy number in undifferentiated cells. Additionally, studies that have investigated the mechanism of degradation of the PDZ proteins in-vitro have reached opposite conclusions as to whether E6AP is the ubiquitin ligase that promotes proteosomal degradation.

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PROFORMA

1. Case study No.: Date:

   IP/OP No.:

2. Patient Name: Age:

3. Address:

4. Education: Yes/No Specification:

5. Working women: Yes / No

   If Yes, state the occupation:

   If No, state the mode of income for family:

6. Married : Yes / No Age when married :

7. No. of pregnancies :

8. Type of testing undergone : HPV testing / Cytologic testing / VIA

9. Treatment given already : Chemotherapy / Radiotherapy / None

10. Clinical CIN grade : I / II / III / IV / could not be assessed

11. Cell differentiation : Well differentiated / moderately differentiated / poorly differentiated / undifferentiated / unknown

12. Samples : Blood / Tumour tissue / Normal tissue

13. Abnormal findings / symptoms :

14. Sample collected by:

15. Consent from patient:

Signature

INFORMATION TO PARTICIPANTS

Title: Over expression of HPV E6/E7 Oncoprotenins in a marker of progression to cervical cancer"

Principal Investigator: **Dr. D. KAMALI, MBBS,DGO**
Post Graduate in M.S., Obstetrics and Gynaecology
Institute of Social Obstetrics
Kasturba Gandhi Hospital
Madras Medical College
Chennai - 600003

Name of Participant:

Site: **Institute of Social Obstetries**
Kasturba Gandhi Hospital
Madras Medical College
Chennai – 600003

You are invited to take part in this research/ study/procedures/tests. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

What is the purpose of research?

Cervical Cancer is a common disorder characterized by_ Bleeding per vaginum/ white discharge PV These symptoms may last for 2 to 3 years.. We want to test the efficacy and safety of a new lab test in this disease/condition.

We have obtained permission from the Institutional Ethics Committee.

The study design

All patients in the study will be divided into two groups (if there are groups). You will be assigned to either of the groups. [Explain briefly].

Study Procedures

The study involves evaluation of genes/ lab test) for which we will be monitoring your symptoms The planned scheduled visits involve visits at three to six months at

once depends upon on HPE report or cervi plus report and after your initial visit. You will be required to visit the hospital 3 number of times during the study.

At each visit, the study physician will examine you. Some [blood / urine / other] tests will be carried out at each visit. [… … ml of blood will be collected at each visit. Blood collection involves prick with a needle and syringe.] These tests are essential to monitor your condition, and to assess the safety and efficacy of the treatment given to you.

In addition, if you notice any physical or mental change(s), you must contact the persons listed at the end of the document. [You will be required to return unused study medicines when you report for your scheduled visits. This will enable correct assessment of the study results. (**for drug studies**)]

You may have to come to the hospital (study site) for examination and investigations apart from your scheduled visits, if required. (In sample collection studies eg tissues/blood etc and analysis of gene studies - you must give information on the study and purpose of the tests)

Women of childbearing potential

You may t participate if you are pregnant, breastfeeding a child, or if you are of childbearing potential and not practicing effective methods of contraception **(for studies/procedures which may no harm to the fetus).**

Possible risks to you: Nil

Possible benefits to you -: Yes

The objective of screening t is to reduce the incidence and mortality from cerivical cancer. Even a single smear in a life time, if appropriately timed, will produce some benefits. If extended only to high risk group, the mortality from the cancer deaths will still be reduced to 60 percent.

Possible benefits to other people

The results of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, Institutional Ethics Committee and any person or agency required by law like the Drug Controller General of India to view your data, if required.

The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

How will your decision to not participate in the study affect you?

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. You will be taken care of and you will not loose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start?

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. However, it is advisable that you talk to the research team prior to stopping the treatment/discontinuing of procedures etc.

Signature of Investigator **Signature of Participant**

Date Date:

INFORMED CONSENT FORM

Title: "Over expression of HPV E6/E7 Oncoprotenins in a marker of Progression to cervicalcaner"

Name of the Investigator: Dr.Kamali

Name of the Participant:

Name of the Institution: Institute of Social Obstetries
Kasturba Gandhi Hospital
Madras Medical College
Chennai - 600003

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in this study.

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have informed the investigator of all the treatments I am taking or have taken in the past months/years including any native (alternative) treatments.
6. I have been advised about the risks associated with my participation in the study.*****
7. I agree to cooperate with the investigator and I will inform him /her immediately if I suffer unusual symptoms. *****
8. I have not participated in any research study within the past 24 month(s). *****
9. I have not donated blood within the past 6 months. . *****
10. I am aware of the fact that I can opt out of the study at any time without having to give any reasoned this will not affect my future treatment in this hospital. *****
11. I am also aware that the investigators may terminate my participation in the study at any time, for any reason, without my consent. *****

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC if required.
13. I understand that my identity will be kept confidential if my data are publicly presented.
14. I have had my questions answered to my satisfaction.
15. I consent voluntarily to participate in the research/study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form, I attest that the information given in this document has been clearly explained to me and understood by me. I will be given a copy of this consent document.

For adult participants

1. Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____ Date_____

2. Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

For observational studies or those in which patients tissue, body fluids are collected for any kind of analysis, **points 6,7,8,9,10,11** may be excluded in such cases.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr.Kamali
Postgraduate M.S.(OG)
Madras Medical College
Chennai 600 003

Dear Dr.Kamali,

The Institutional Ethics Committee has considered your request and approved your study titled **"Overexpression of HPV E6/E7 Oncoproteins is a marker of progression to cervical cancer" No.15042015.**

The following members of Ethics Committee were present in the meeting held on 07.04.2015 conducted at Madras Medical College, Chennai-3.

1.	Prof.C.Rajendran, M.D.,	: Chairperson
2.	Prof.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3.	Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.B.Vasanthi, M.D., Prof. of Pharmacology, MMC	: Member
5.	Prof.P.Ragumani, M.S., Professor of Surgery, MMC	: Member
6.	Prof.S.Baby Vasumathi, Director, Inst. Of O&G, MMC	: Member
7.	Prof.K.Ramadevi, Director, Inst.of Biochemistry, MMC	: Member
8.	Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9.	Prof.K.Srinivasagalu, M.D.,Director, I.I.M. MMC, Ch-3	: Member
10.	Thiru S.Rameshkumar, B.Com., MBA	: Lay Person
11.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

14/5/15

Member Secretary, Ethics Committee

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CHENNAI-3.

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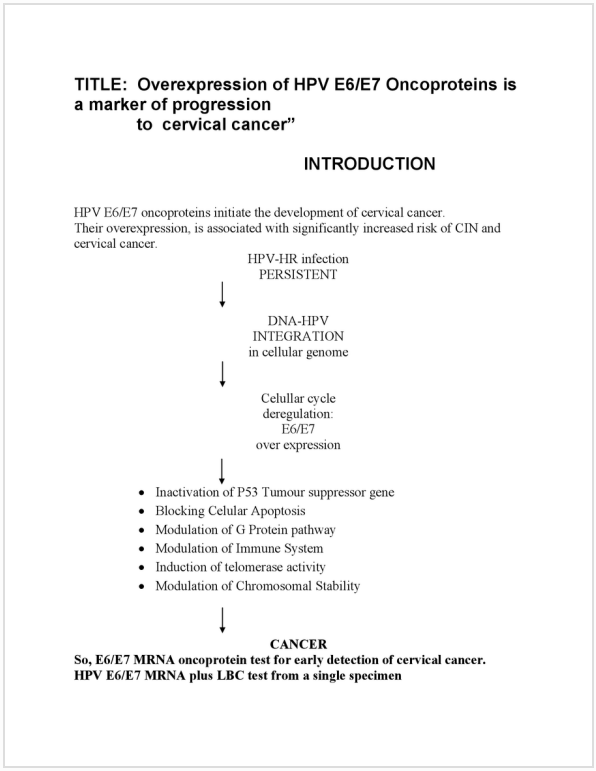

TITLE: Overexpression of HPV E6/E7 Oncoproteins is a marker of progression to cervical cancer"

INTRODUCTION

HPV E6/E7 oncoproteins initiate the development of cervical cancer.
Their overexpression, is associated with significantly increased risk of CIN and cervical cancer.

HPV-HR infection
PERSISTENT

↓

DNA-HPV
INTEGRATION
in cellular genome

↓

Celullar cycle
deregulation:
E6/E7
over expression

↓

- Inactivation of P53 Tumour suppressor gene
- Blocking Celular Apoptosis
- Modulation of G Protein pathway
- Modulation of Immune System
- Induction of telomerase activity
- Modulation of Chromosomal Stability

↓

CANCER

So, E6/E7 MRNA oncoprotein test for early detection of cervical cancer.
HPV E6/E7 MRNA plus LBC test from a single specimen

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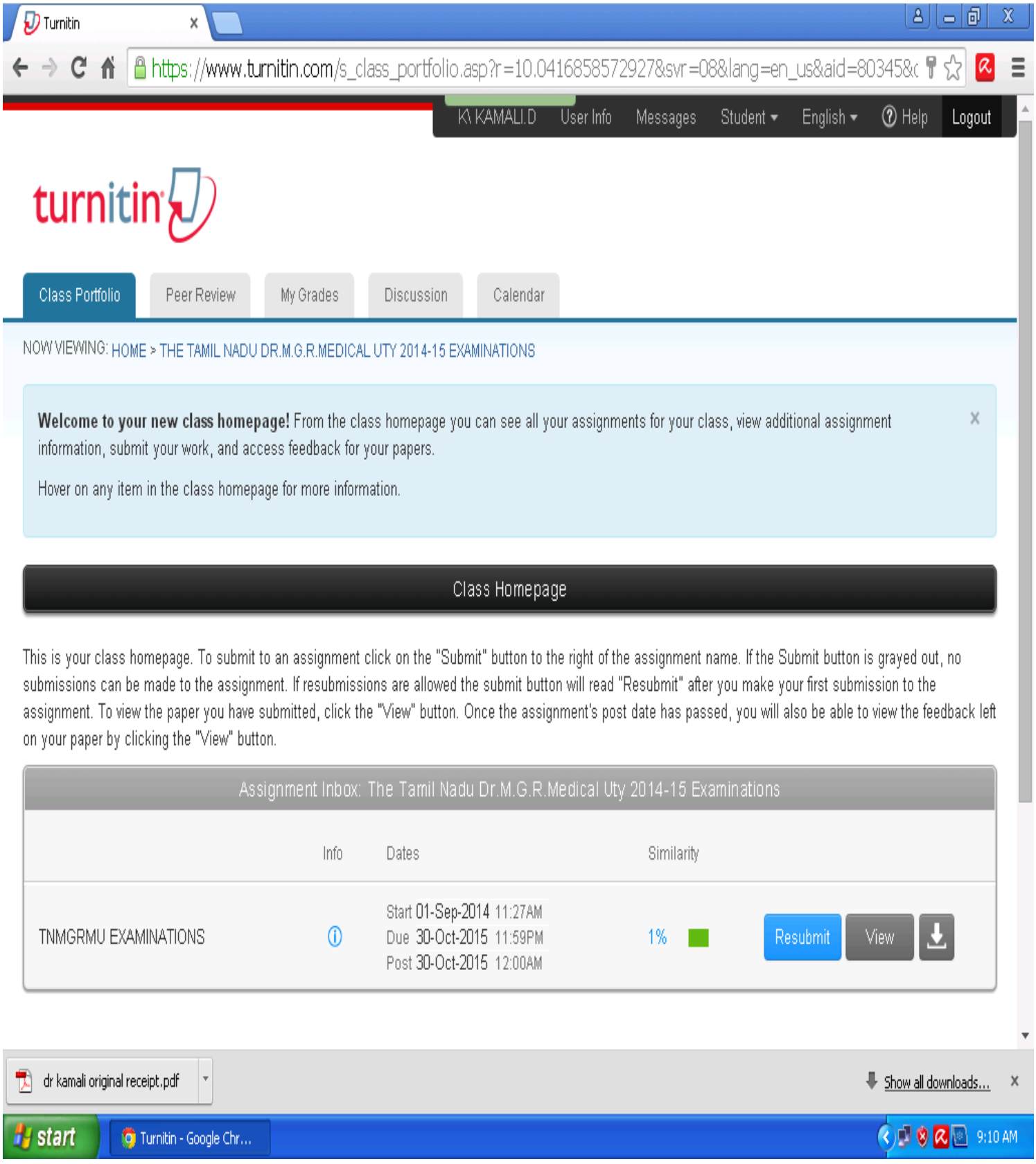
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